Green Fluorescent Protein

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I. Abstract

The green fluorescent protein is a bioluminescent protein that is commonly found in the Aequorea Victoria jellyfish and most recently found in the first ever reptile, the Hawsbill Sea Turtle. The 3D modeling project helped with the overall understanding of proteins and even 3D printing. At the end of the project, a 3D model of the GFP protein was created and modeled in front of the class.

II. Introduction

A protein is "a biologically functional molecule consisting of one or more polypeptides folded and coiled into a specific three dimensional structure" (Reece). To gain a better understanding of a protein, one should look into the history and development of a protein. Protein comes from the Greek word *proteios*, meaning *first* or *primary.* They account for more than 50% of the mass of most cells. Proteins are instrumental in almost every aspect of what an organism does. Some proteins speed up chemical reactions while others play roles in defense, storage, transport, cellular communication, movement, or structural support (Reece). When it comes to the structure of proteins, there are four different types of structures: primary, secondary, tertiary, and quaternary. Primary structures are a linear representation of amino acids in the chain. Secondary structures builds off of the primary structure but it forms simple structures that represent the polypeptide. Tertiary structures are the three dimensional representation of the polypeptide molecules. Quaternary structures are another form of a three dimensional structure but it represents how everything combines together to form the protein. Aside from the structure, there are also classes of proteins. According to Regina Bailey, a biology expert on About.com/Education, "there are two general classes of protein molecules: globular proteins and fibrous proteins. Globular proteins are generally compact, soluble, and spherical in shape. Fibrous proteins are typically elongated and insoluble". Now, where do proteins come from? Proteins are synthesized in individual cells through a process called translation. During this process, genetic code (DNA) is generated from the nucleus of the cell and then free-floating ribosomes translate this code into polypeptide chains. Then these chains are modified to become fully functioning proteins where then the Golgi Apparatus then sends the proteins to where they are needed throughout the cell or body.

III. Methods/Research

In order to complete the project throughout the semester, we came up with deadlines for ourselves to have certain aspects of the project completed by a specific timeframe. We drafted an outline with questions from ourselves and peers that we felt were important to the understanding of our protein. For this project, our team researched many different aspects of green fluorescent protein, including: the genetic makeup of the protein, where it is found, what it does, the structure of the protein, and any hydrophobic or hydrophilic characteristics. We found that, GFP is a protein mostly found in marine life, and reacts to ultra violent lights in which makes it illuminate a neon green color. GFP is used as a defense mechanism against predators for most organisms and can also be used to lure prey or find partners (Castel 1). GFP is also used as a cell and protein marker in many experiments. “As a tag, GFP usually reflects levels of gene expression, and as an indicator GFP fluorescence is mostly used to study protein-protein interactions” (Castel 5)[CL1] . Green Fluorescent Protein is most commonly found in Aequorea Victoria jellyfish, but can also be found in fireflies, glowworms, certain fungi and bacteria. It has both hydrophobic and hydrophilic characteristics [CL2] and consists of 238 amino acids. The Green Fluorescent Protein is hydrophobic and when placed in a high salt concentration the bacteria goes through the HIC columns and HIC matrix binds GFP molecules. Once that happens the salt concentration needs to be lowered so that the buffer causes the Green Fluorescent Protein to elute the column in pure form. [citation?][CL3] “It has a unique soda can shape. Eleven beta-strands make up the beta-barrel and an alpha-helix runs through the center. The chromophore is located in the middle of the beta-barrel, it is occasionally referred to as the ‘light in the can’” (Green Fluorescent Protein). “The chromophore of GFP is responsible for its fluorescence. It has the following structure where the R groups are the first 64 and last 170 residues of GFP. GFP catalyzes the formation of its own chromophore. It is proposed that Arg96 plays a crucial role in this catalysis”(Green Fluorescent Protein).[CL4] It is similar to bioluminescence since bioluminescence is the production of light by an organism. GFP produces both color and light. The structure of GFP is capable of changing and there are various mutants that are categorized into seven classes based on spectral properties. “The environment inside the cylinder explains the effects of many existing mutants of GFP and suggests which side chains could be modified to change the spectral properties of GFP” (Yang, Fan, Larry Moss, and George Phillips, Jr.).

IV. Interesting Current Events

The Green Fluorescent Protein, also known as Bioluminescence, is commonly found in many aquatic creatures but on a recent National Geographic expedition, the first bioluminescent sea turtle was discovered. The Hawksbill sea turtle is the first reptile to exhibit bio fluorescence. David Gruber, of City University of New York, went to the Solomon Islands in July of 2015 to film bioluminescent sharks and coral reefs but finding the turtle was an even bigger feat. The Hawksbill sea turtle glowed with green and red but scientists determined that the red came from the coral reef in the surrounding environment; however, the green came solely from the turtle itself. Gruber stated that it was too soon to know how the turtle has the ability to fluoresce but his prediction is that the turtle had to adapt to its surroundings (the coral reef) to camouflage itself amongst the glowing reef. Since this discovery was so recent, biologists have yet to truly determine how the turtle received the Green Fluorescent Protein but are working hard to solve this mystery

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[INSERT HYPOTHESIS]

The Green Fluorescent Protein is also helping significantly with medical research. Sanford at the Tech Understand Genetics put the glowing protein into cancer cells of animals, which causes those cells to glow. This helps because medical researchers can watch how cancer moves through the body. They do this by adding the Green Fluorescent Protein to the cancer cells that are growing in a dish. Afterwards, they place cancer cells inside the animal. Now medical researchers can track certain cancers and see where it spreads throughout the body. They are hopeful to find ways to stop the cancer before it develops further into the body. The Green Fluorescent Protein is not just a protein that helps animals glow but also a significant tool in the medical field.

V. Modeling

The structure for a Green Fluorescent Protein is barrel and cylinder like. Chromophore is responsible for the fluorescence in the molecule. The Primary structure of the protein is Amino ($NH3+$) to Carboxy ($COO-$).

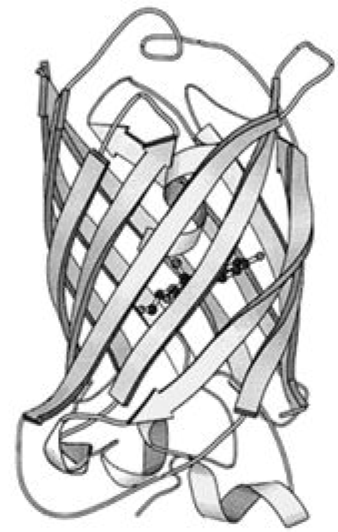

Figure 1: The tertiary structure of GFP. The central darker circles represent the chromophore, while the long flat sheets represent the barrel surrounding it. (King, GFP Structure-3)

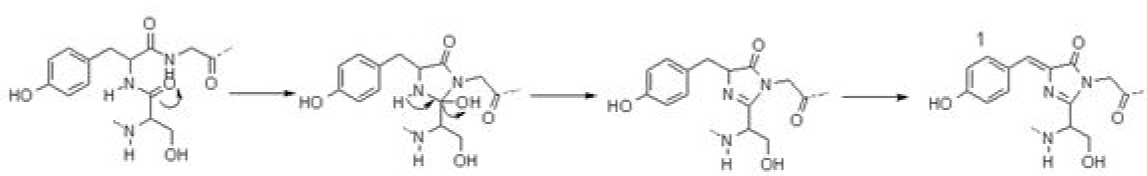

Figure 2: The mechanism for formation of the chromosphere of GFP. Molecule 1 is the final structure of the chromosphere. The first two steps happen automatically without outside input, however, the final step requires atmospheric oxygen. The dotted lines represent bonds to the rest of the GFP protein. (King, Scheme 2)

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     M  S  K  G  E  E  L  F  T  G  V  V  P  I  L  V  E  L  D  G
  1 ATGAGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGT
     D  V  N  G  H  K  F  S  V  S  G  E  G  E  G  D  A  T  Y  G
 61 GATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGA
     K  L  T  L  K  F  I  C  T  T  G  K  L  P  V  P  W  P  T  L
121 AAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTT
     V  T  T  F  S  Y  G  V  Q  C  F  S  R  Y  P  D  H  M  K  Q
181 GTCACTACTTTCTCTTATGGTGTTCAATGCTTTTCAAGATACCCAGATCATATGAAACAG
     H  D  F  F  K  S  A  M  P  E  G  Y  V  Q  E  R  T  I  F  F
241 CATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTC
     K  D  D  G  N  Y  K  T  R  A  E  V  K  F  E  G  D  T  L  V
301 AAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTT
     N  R  I  E  L  K  G  I  D  F  K  E  D  G  N  I  L  G  H  K
361 AATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAA
     L  E  Y  N  Y  N  S  H  N  V  Y  I  M  A  D  K  Q  K  N  G
421 TTGGAATACAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGA
     I  K  V  N  F  K  I  R  H  N  I  E  D  G  S  V  Q  L  A  D
481 ATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGAC
     H  Y  Q  Q  N  T  P  I  G  D  G  P  V  L  L  P  D  N  H  Y
541 CATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTAC
     L  S  T  Q  S  A  L  S  K  D  P  N  E  K  R  D  H  M  V  L
621 CTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTT
     L  E  F  V  T  A  A  G  I  T  H  G  M  D  E  L  Y  K  *
681 CTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAA
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Figure 3: GFP consists of 238 amino acids. This figure shows the sequence of the amino acids in the GFP protein. (Castel)

VI. Conclusion

Works Cited

Bailey, Regina. "What Are Proteins?" *About.com Education*. About.com, n.d. Web. 27 Oct. 2015.

Castel, Rob. "Green Fluorescent Protein." Ed. Holger Lill. *SpringerReference* (2011): n. pag. Vrije Universiteit Amsterdam, May 2003. Web. 29 Oct. 2015.

Digital image. *Royal Society of Chemistry*. N.p., n.d. Web. 29 Oct. 2015. <http://www.rsc.org/images/FEATURE-NOBEL-350_tcm18-136787.jpg>.

"Green Fluorescent Protein - GFP Structure." *Green Fluorescent Protein - GFP Structure*. N.p., n.d. Web. 07 Nov. 2015.

King, Timothy, and Paul May. *GFP Structure-3*. Digital image. *Green Flourescent Protein*. University of Bristol, n.d. Web. 29 Oct. 2015. <http://www.chm.bris.ac.uk/motm/GFP/GFP-3structure.gif>.

King, Timothy, and Paul May. *Scheme 1*. Digital image. *Green Fluorescent Protein*. University of Bristol, n.d. Web. 29 Oct. 2015. <http://www.chm.bris.ac.uk/motm/GFP/mechanism.gif>.

Nobel For Chemical Jellyfish Lights-2. Digital image. *Northwestern.edu*. North Western University, n.d. Web. 29 Oct. 2015. <http://i1-news.softpedia-static.com/images/news2/Nobel-For-Chemical-Jellyfish-Lights-2.jpg>.

Reece, Jane B., Lisa A. Urry, Michael L. Cain, Steven Alexander Wasserman, Peter V. Minorsky, and Rob Jackson. *Campbell Biology*. 10th ed. N.p.: Pearson Education, 2014. Print.

Stephens, David. A pseudocoloured image of the jellyfish Aequorea victoria from which Green Fluorescent Protein was isolated. The pseudocolouring illustrates the diverse colour palette of GFP variants that we now have available. The image is a photograph taken by David Stephens at Monterey Bay aquarium in 2003. There is only one jellyfish, this image is a montage. Digital image. *Stephens Lab*. Worldpress.com, 19 Dec. 2012. Web. 29 Oct. 2015. <https://stephenslab.files.wordpress.com/2012/12/aequorea-spectra.jpg>.

Yang, Fan, Larry Moss, and George Phillips, Jr. "The Molecular Structure of Green Fluorescent Protein." *The Molecular Structure of Green Fluorescent Protein*. N.p., n.d. Web. 17 Nov. 2015.

[CL1]Shouldn't use 2 references back to back. There needs to be our own thoughts inbetween references explaining what is going on.

[CL2]What makes it both? What in the protein allows it to be hydrophilic and hydrophobic?

[CL3]Citation?

[CL4]Again, 2 references back to back…